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Running title: Beryllium Analysis by Accelerator Mass Spectrometry

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Abstract

A method using accelerator mass spectrometry (AMS) has been developed for quantifying attomoles of beryllium (Be) in biological samples. This method provides the sensitivity to trace Be in biological samples at very low doses with the purpose of identifying the molecular targets involved in chronic beryllium disease. Proof of the method was tested by administering 0.001, 0.05, 0.5 and 5.0 µg ^{9}Be and ^{10}Be by intraperitoneal injection to male mice and removing spleen, liver, femurs, blood, lung, and kidneys after 24 h exposure. These samples were prepared for AMS analysis by tissue digestion in nitric acid, followed by further organic oxidation with hydrogen peroxide and ammonium persulfate and lastly, precipitation of Be with ammonium hydroxide, and conversion to beryllium oxide at 800°C. The ^{10}Be/^{9}Be ratio of the extracted beryllium oxide was measured by AMS and Be in the original sample was calculated. Results indicate that Be levels were dose-dependent in all tissues and the highest levels were measured in the spleen and liver. The measured ^{10}Be/^{9}Be ratios spanned 4 orders of magnitude, from 10^{-10} to 10^{-14}, with a detection limit of 3.0×10^{-14}, which is equivalent to 0.8 attomoles of ^{10}Be. These results show that routine quantification of nanogram levels of Be in tissues is possible and that AMS is a sensitive method that can be used in biological studies to understand the molecular dosimetry of Be and mechanisms of toxicity.

Introduction

Inhalation exposure to Be from a variety of manufacturing processes can cause numerous health problems, including the granulomatous condition chronic beryllium disease (CBD) (1). CBD is a debilitating, progressive and potentially fatal disease that predominantly affects the lung (2). Pathogenesis involves development of an immunologic response, followed by a chronic inflammatory response and associated histological changes, physiological impairments, and in some cases, death (3). Current research suggests that the mechanism by which CBD develops is that Be mediates the binding of an antigenic peptide to the human leukocyte antigen (HLA)-DP heterodimers formed on the cell surface of antigen-presenting cells (macrophages, B lymphocytes, and other cells). This complex is recognized by T Cell Receptors on the cell surface of specific T cell clones and this antigen recognition process activates these specific T cells, resulting in an autoimmune response (4).

The inflammation caused by CBD is typically controlled by using corticosteroids (1, 5). However, preventing the recurrence of symptoms requires lifelong steroid treatment and other supportive treatments including oxygen, bronchodilators and immunizations against respiratory pathogens. Understanding the inflammatory and antigen-specific immune features of the disease can assist in the development of new pharmacologic treatments (6). The objective of this study is to develop unique methodology that enables very sensitive measurements of Be in biological samples that will further the understanding of the cellular and molecular mechanisms responsible for CBD in humans.

^{9}Be is the naturally occurring isotope while ^{7}Be and ^{10}Be are two radioisotopes. This methodology uses ^{10}Be (half-life = 1.5. x 10^{6} y) as a tracer that is quantified by accelerator mass spectrometry (AMS). The most well known application of AMS is in carbon dating, but many applications in the biological and earth sciences have also been developed (7, 8). In particular, ^{10}Be is routinely analyzed by AMS for dating of geologic samples (7, 8, 9).

In AMS, atoms of the isotope of interest are individually counted after passing through a spectrometer. For long-lived isotopes, such as ^{10}Be, decay counting methods are limited in sensitivity by the small number of atoms which decay in a reasonable measurement time. By counting atoms instead of decays, AMS sensitivity is not affected by the decay rate, and can therefore be orders of magnitude more sensitive for half-lives greater than a few years. For many of the isotopes typically measured by AMS, sensitivity is sub-attomole, corresponding to activities of nCi to fCi (10, 11). AMS sensitivity of ^{10}Be is ~0.5 attomoles, or ~1 x 10^{-19} Ci.

Use of ^{10}Be offers a sensitive alternative to other methods for the study of the disposition of beryllium in biological systems. Analytical determination of ^{9}Be is not sufficiently sensitive enough for direct tracking of Be at levels relevant to low-dose exposure. The shorter-lived radioisotope ^{7}Be (half-life 53.3 d) produces a readily measured gamma on decay and sensitivity for decay counting of ^{7}Be, in atoms per sample, is similar to that of AMS measurements of ^{10}Be, however, the specific activity of ^{7}Be is ~10^{7} times higher. For typical low dose exposures, this means the handling of Curie-sized quantities versus sub-μCi of ^{10}Be to obtain similar sensitivity. The lower activities required for ^{10}Be has obvious savings in terms of ease of handling,

experimenter safety, safety of the experimental subject, waste disposal, transport of samples, and sample preparation. In addition, the long half-life of ^{10}Be enables the possibility of long-term (weeks to years) studies without loss of sensitivity.

The objective of this study was to develop a ^{10}Be tracer methodology for following nanogram levels of beryllium in biological samples. This sensitivity will allow future studies on the cellular and molecular mechanisms responsible for CBD in humans and is useful for tracing Be in a biological system with the purpose of defining the molecular targets of Be at very low doses. The experiments were designed as a comparison to a ^{7}Be study in mouse tissues conducted by Sakaguchi (12) to validate our AMS method and determine a Be dose-response in mouse tissues.

Experimental Procedures

Caution: *Be is a suspected human carcinogen and mutagen and should be handled with care.*

Materials. A supply of high enrichment ^{10}Be was obtained from the Medical Radioisotope Program, Los Alamos National Laboratory (LANL), Los Alamos, New Mexico. The ^{9}Be standard was 1000 ppm $Be(NO_3)_2$ AAS grade in weak nitric acid (VWR Scientific Products, West Chester, PA). Niobium powder (99.99%; -325 mesh) was purchased from Alfa Aesar (Ward Hill, MA). All other chemical reagents were of analytical grade.

Preparation of Isotopic Beryllium Dosing Solutions. Isotopic purity in the original stock solution from LANL was determined by atomic absorption spectrophotometry (AAS), liquid scintillation counting (LSC), and gas scintillation proportional counting (GSPC). Total Be content (^{10}Be and ^{9}Be) was measured by graphite furnace AAS (Perkin Elmer, Shelton, CT). ^{10}Be radioactivity was measured by counting duplicate samples by LSC using a Pharmacia Wallac 1410 liquid scintillation counter (Gaithersburg, MD) and a Gamma Products G5000 gas proportional counter (Palo Hills, IL). The LSC results were calibrated using a ^{90}Sr standard (β endpoint energy 0.546 MeV), relative to the ^{10}Be β endpoint energy of 0.556 MeV. The LANL standard solution was serially diluted with 1000 ppm ^{9}Be AAS standard to obtain stock solutions containing 1.0 mg/mL ^{9}Be with $^{10}Be/^{9}Be$ ratios ranging from 2.2×10^{-6} to 1.1×10^{-12}. The ^{9}Be content of these stock solutions was verified by AAS as 1.09 ± 0.08 mg/mL. The 0.001, 0.05, 0.5, and 5.0 µg Be dosing solutions were prepared by dilution of the stock solutions in 0.1 M phosphate buffer, pH 7.9.

AMS standards, with $^{10}Be/^{9}Be$ ratios between 1.1×10^{-9} and 1.1×10^{-14} were prepared by serial dilution of the LANL standard in 0.5 N HNO_3. The LLNL standards were normalized to a ^{10}Be ICN standard prepared by K. Nishiizumi (13, 14).

Biological Sample Matrixes. The sample preparation method was initially tested by spiking triplicate 200 mg liver samples with 0.1, 1.0, and 10 µg Be/g tissue and measured by AAS. This method was then validated for AMS using liver, lung and urine samples. Triplicate 100 mg liver and lung tissue and 2.0 mL of urine samples were spiked with 0.1, 1.0, and 10 µg Be/g tissue with different $^{10}Be/^{9}Be$ ratios obtained by diluting the LANL stock solutions described earlier. The samples were prepared for AMS analysis with 1.0 mg ^{9}Be carrier added to them. Table 3 summarizes the ^{9}Be dose concentration and $^{10}Be/^{9}Be$ ratio added to each tissue and the expected $^{10}Be/^{9}Be$ ratio after AMS measurement. Triplicate samples of the LLNL and LANL standards were prepared and analyzed with the tissue samples having three different $^{10}Be/^{9}Be$ ratios (10^{-10}, 10^{-11}, 10^{-12}).

The intersample coefficient of variation (CV) was determined by measuring the $^{10}Be/^{9}Be$ ratio in 200 mg of ten separate spleens, lungs and homogenized livers dosed with 0.05 µg ^{9}Be (2.2×10^{-6} $^{10}Be/^{9}Be$ ratio) and calculating the ng ^{9}Be/g wet weight of tissue (group#1; Table 1). The intrasample CV was determined by pooling the remainder of the homogenized livers and processing 10 aliquots of 200 mg each and measuring the samples by AMS.

Laboratory Animal Dosing and Sample Collection. This study was approved by the LLNL Institutional Animal Care Committee in accordance with the guidelines set forth in the National Institutes of Health Guide for the Care and Use of Laboratory

Animals. Male ICR mice (30 g), 6-7 weeks old, were obtained from Harlan Sprague Dawley Inc. (Madison, WI). Animals were housed in individual stainless steel cages on a 12 h light/dark cycle at room temperature (22°C) and given water and food *ad libitum*. Dosing solutions were administered in 0.1 M phosphate buffer pH 7.9 with animals receiving 0.2 mL by intraperitoneal injection.

The experimental treatments for the two groups of mice are summarized in Table 1. Group 2 consisted of 20 mice (5 animals per dose) that received 0.001, 0.05, 0.5 and 5.0 µg Be solution with the same $^{10}Be/^{9}Be$ ratio (2.2×10^{-6}) to measure a Be dose-response and distribution in different tissues. The third group of 4 mice received 0.5 µg Be with a $^{10}Be/^{9}Be$ ratio of 2.2×10^{-6} to measure total Be in a whole animal and in urine. Each group had 3 mice as controls receiving 0.1 M phosphate buffer pH 7.9 by intraperitoneal injection. The mice were euthanized by carbon dioxide asphyxiation 24 h after dosing. Blood was removed by cardiac puncture and collected in eppendorf tubes containing 50 units heparin/mL of blood. Table 1 lists the tissues that were removed for each dose group and stored at -70°C and average tissue wet weights are given in Table 2.

Process and carrier blanks were treated like the tissue samples but did not contain any ^{10}Be. The $^{10}Be/^{9}Be$ ratios of 1.0 mg ^{9}Be standard were measured in the carrier blanks and the process blanks (N=3) contained 0.25 – 1.0 mg carrier and followed the same sample preparation steps as the dosed tissues.

AMS Sample Preparation. Sample preparation for AMS analysis consists of Be extraction by acid digestion after addition of a carrier, followed by precipitation of Be and oxidation to BeO (15). The ^{9}Be AAS standard is used as the chemical carrier and acts as an internal isotope dilution. Whole mice were placed in 250 mL Teflon® beakers

(Savillex Corp., Minnetonka, MN) and 1.0 mg Be standard was added as carrier with 100 mL HNO_3 (70% v/v) and heated to 100°C. A total of 100 mL hydrogen peroxide (30% v/v) was added 25 mL at a time to prevent the solution from bubbling over. 10 mL HF (49% v/v) was added to keep the Be in solution. The tissues were further oxidized by adding 100 mL of a 4.0% (w/v) solution of ammonium persulfate in water. The samples were repeatedly taken close to dryness to eliminate organics. Liver and kidneys were processed in a similar manner but with 10 mL of HNO_3 and hydrogen peroxide followed by 10 mL of 4% ammonium persulfate. All other tissues (lung, spleen, blood, femurs, urine) were treated with 2.0 mL each of HNO_3, hydrogen peroxide, and ammonium persulfate. All tissues received 0.25 mg Be carrier except for the whole mouse and 5.0 µg dosed liver samples which received 1.0 mg. In addition, all groups of samples had triplicate process blanks containing 250 µg carrier and were treated like the tissues. After treatment with ammonium persulfate, water was added to the sample to a final volume of 4 mL in 15 mL conical polycarbonate centrifuge tubes for the tissues and to a final volume of 40 mL for the whole mice. The spleen and liver samples dosed with 5.0 µg Be were diluted prior to Be extraction by adding additional 500 and 1000 µg Be carrier, respectively, to a 0.5 mL aliquot. The whole mouse samples were also diluted by adding 1000 µg carrier to a 1.0 mL aliquot.

The femurs were further treated with an ammonium molybdate solution to extract calcium from the sample (16). This solution contained 100 g ammonium molybdate in 400 mL of ultrapure water and 80 mL of concentrated ammonium hydroxide. One volume of the ammonium molybdate solution was combined with two volumes of a 28% nitric acid (v/v) solution immediately before use and 10 mL was added to 3 mL of the

digested femur sample and left overnight. The samples were centrifuged as described earlier and passed through a 0.45 µM Uniflo® sterile filter (Schleicher & Schuell BioSciences Inc., Keene, NH). A small amount (0.5 mL to 1 mL) of equal parts of ammonium hydroxide (% v/v) and ultrapure water was then added to the filtered supernatant (pH 0-1) to cause a secondary precipitation, but keep the Be in solution. The samples were centrifuged and filtered again.

Be was extracted from the samples by adding 1-2 mL of equal parts of ammonium hydroxide and ultrapure water resulting in a pH of 8-9. The samples were centrifuged after 24 hours for 10 min at 1500 g. The supernatant was discarded and the precipitated Be was transferred to a quartz crucible for the oxidation step. The extraction steps employed for preparation of Be standards and blank solutions were identical to those employed with the samples. The beryllium blanks were prepared using 250 µg of the AAS ^{9}Be standard.

After the Be extraction steps, the pellet (sample, standard or blank) was transferred to a 17 mm x 9 mm quartz crucible (Ace Glass Company, Louisville, KY). The crucible was dried in an oven at 85-95 ºC for 24 h. The sample was gradually heated to 100-110 ºC to remove any residual moisture, and then increased to 800ºC at 5 ºC/min. After baking, the crucibles were cooled and capped.

The final step for preparing the sample for AMS analysis was to add 2 mg of niobium to each crucible. The niobium is used to reduce the amount of required Be carrier, which increases the measured ratios and secondly, niobium greatly enhances Be negative ion production in the accelerator (Fig. 1). Sample homogenization and packing into holders for AMS analysis was done in a Plexiglas box in a hood to reduce sample

cross contamination. The sample and niobium were homogenized inside the crucible using a #56 drill stem that had been washed with acetone, rinsed with ultra-pure water and dried prior to use (Precision Twist Drill Company, Crystal Lake, IL). After homogenization, the sample was transferred to an aluminum sample holder that was previously washed in acetone and rinsed in ethyl acetate. The acetone removes any machining oils, and the ethyl acetate allows the powdered sample to slide into the cathode orifice easily. Once the sample was in the aluminum sample holder, it was packed using the #56 drill stem and a hammer.

^{10}Be AMS Measurement. AMS measures the $^{10}Be/^{9}Be$ ratio in the prepared sample. Measurements are made in quasi-simultaneous injection mode, in which $^{10}Be^{3+}$ counts are measured for 300 ms, followed by measurement of $^{9}Be^{3+}$ current for 5 ms. This rapid cycling provides an implicit internal correction for time-dependent drifts of the ion source output and this cycle is repeated for the duration of the measurement. Sample $^{10}Be/^{9}Be$ ratios were determined by comparing the ratio of ^{10}Be counts to ^{9}Be integrated charge to the same ratio for a standard. Samples were typically counted for 5 mins or 2 times for a counting precision of 0.5-2%.

The final ng ^{9}Be/g wet tissue weight was determined using the following formulas:

$^{10}Be_{(atoms)}$/tissue weight$_{(g)}$= (measured $^{10}Be/^{9}Be$ ratio ***** Be carrier/$9.012_{(Be\ MW)}$)*****
(6.02×10^{23} ***** tissue weight$_{(g)}$)

ng ^{9}Be/g wet tissue weight = ($^{10}Be_{(atoms)}$/tissue weight$_{(g)}$/ ^{10}Be dose$_{(atoms)}$) ***** (^{9}Be dose$_{(atoms)}$/ (9.012 ***** 6.02×10^{23})) ***** 10^{9}

Statistics. The AMS limit of detection (LOD) for the biological tissues was calculated from the sum of the mean $^{10}Be/^{9}Be$ ratios of the control tissues plus three times

the SD of the mean (N=18). The instrument LOD has been previously described as 5.0 x 10^{-15} (7). The coefficient of variation (CV) is defined as the precision or closeness of individual measurements of replicate samples and was expressed as a percent. The CV was calculated by dividing the SD by the mean value. Accuracy describes the closeness of the measured values to the expected calculated values.

Simple linear regression was used for the AMS measurements on six tissues taken from each of nineteen mice. Analyses involved regressing $\log_{10}$ Be measured on $\log_{10}$ Be dose. The data were fit to a model of the form

$$\log_{10} y = \alpha + \beta \times \log_{10}\text{Dose} + \varepsilon$$

using separate intercept and slope terms (α_i, β_i) for each tissue and ε is the error term or the distance from the actual value of $\log_{10} y$ from the regression line. Differences between the estimated intercepts and slopes for each tissue were compared using *t*-tests, and noting those comparisons that were not significant at the 0.05 level (Table 6). The threshold used for detecting pairwise differences was *Tukey's Honest Significance Difference* procedure (17).

Results

Description of AMS for Measuring $^{10}Be/^{9}Be$ Ratios. $^{10}Be/^{9}Be$ ratios were measured using the tandem Van de Graff accelerator at LLNL. A diagram of the accelerator is shown in Figure 1. AMS uses differences in charge/mass ratio and in rates of energy loss to separate the isotopes of beryllium (and other elements) so that ions of the rare isotope (in our case,^{10}Be) can be counted (7). The sample is bombarded in the ion source by cesium ions, causing negatively charged ions to be produced from the sample. ^{9}Be current is measured after the ions pass through a high-energy mass spectrometer. The negative ions are stripped to positive charge state at the terminal of the accelerator and further accelerated. ^{10}Be ions lose energy in the gas ionization detector and are counted. The rate of energy loss is used to distinguish ^{10}Be from other species.

Instrument Performance and Method Validation. The ^{10}Be activity from the initial LANL standard measured by LSC was 88 ± 1 nCi and is equivalent to 3.7 µg. The total Be (^{9}Be + ^{10}Be) content measured by AAS was 5.3 ± 0.2 µg and therefore, the original LANL stock solution contained 71 ± 7% ^{10}Be which was suitable to conduct biological tracer studies. The measured $^{10}Be/^{9}Be$ ratios by AMS of the diluted LANL and LLNL standards are compared to the expected ratios in Table 3. These $^{10}Be/^{9}Be$ ratios are plotted in figure 2 and illustrate the accuracy, precision, dynamic range, and detection limit of the ^{10}Be AMS measurement. The CV of the standards is less than 2%. The linear dynamic range spanned four orders of magnitude (10^{-14} to 10^{-10}) and an LOD for $^{10}Be/^{9}Be$ ratios from combined control tissues (N=18) was 3.0×10^{-14}.

The $^{10}Be/^{9}Be$ ratios measured by AMS for the liver, lung, and urine samples spiked with 0.1, 1.0, or 10 µg Be/g wet tissue are plotted with the standards in Figure 2 and compared to their expected $^{10}Be/^{9}Be$ ratios in Table 4. The CV among spiked replicate

tissues varied between 2% for urine, 6% for livers and 12% for lungs. There was a strong correlation (r^2= 0.999) between the measured and expected $^{10}Be/^{9}Be$ ratios for all samples measured, indicating good recovery of Be from the sample preparation method and good accuracy of the measurements when compared to the standards. Greater than 90% Be was recovered when liver tissue was spiked with similar concentrations and measured by AAS (data not shown).

The intrasample CV, representing AMS measurements of aliquots of pooled liver tissue was 12% (*N*=10) with an average Be content of 4.3 ± .1 ng/g wet weight. However, the intersample CV among 10 separate dosed liver and spleen tissues was 27% and 13% respectively. The average Be concentration measured in the livers was 4.4 ± 1.2 ng/g wet weight and 8.7 ± 1.1 ng/g wet weight in the spleens. Large variation (>100%) was observed in the lung samples with an average Be concentration of 0.18 ± 0.2 ng/g wet weight.

The $^{10}Be/^{9}Be$ ratios measured for process, carrier, and tissue blanks are summarized in Table 5. Carrier blanks were measured to determine the background $^{10}Be/^{9}Be$ ratios of the ^{9}Be standard while process blanks were measured to check for any cross-contamination of the samples, especially during the acid digestion process. The $^{10}Be/^{9}Be$ ratio of the process and carrier blanks ranged between 12 and 16 x 10^{-15} with a CV that was less than 2%. The tissue blanks had similar ratios but the CV was an average of 4% higher except for the lungs and femurs that were 23 and 51% respectively. The process blanks were as low as the carrier blanks indicating no cross contamination during the multiple sample preparation steps.

Be Dosimetry in Mouse Tissues. The $^{10}Be/^{9}Be$ ratios obtained by AMS analyses of the six different mouse tissues were converted to ng Be/g wet tissue weight and plotted on a log-log scale (Figures 3a and b). The plots indicate linearity and Be dose dependence for all tissues. Table 6 summarizes the differences between intercepts and slopes to compare regression lines. Hence, the lung, blood, liver, and kidney intercept terms were indistinguishable as well as the kidney, femur, and spleen intercept terms. Therefore, intercept terms for lung, blood, and liver were lower (equivalent to 0.009 ng/g tissue) and significantly different from the intercept terms for femurs and spleen (0.3 ng/g tissue). The kidney intercept term was in the middle of the two groups. Similar results were obtained for detecting differences between the slope terms. The femurs, kidneys, blood, and spleen slopes were indistinguishable, as were the blood, spleen, liver, and lung slopes. However, the femur and kidney slopes were lower (equivalent to 6 ng Be/ng Be dose) and significantly different from the liver and lung slopes (17 ng Be/ng Be dose). Blood and spleen slope terms were in the middle. Most of the linear relationships had the same intercept (10 of 15) and the same slope (11 of 15) while only 5 of 15 had different intercepts and 4 of 15 had different slopes.

Be concentrations recovered from whole mouse extract averaged 11 ± 2 ng/g tissue which is equivalent to 71 ± 10% of the initial 0.5 µg dose. Urine collected from the same mice had 6 ± 1 ng/g or 1.4 ± 0.6% of the initial dose.

Discussion

Quantitation of Be in Biological Samples by AMS. This study describes a method for extracting Be from biological samples and measuring the $^{10}Be/^{9}Be$ ratios by AMS so that Be in the samples can be quantitated. AMS sensitivity of control tissues was ~0.8 attomoles of ^{10}Be. At the lowest dose used in this study, it is possible to measure ~2 pg Be/g in mouse liver. For comparison, environmental Be exposures reported for humans, rats and guinea pigs are ~1 ng/g (18). The sensitivity of the AMS method described in this study can also be compared to that of elemental Be methods such as ICP-MS and graphite furnace AAS which have an LOD between 0.1-1 µg Be/L in biological materials such as urine (19, 20).

In future studies, the sensitivity of the present method could be improved by a factor of 100-1000, by preparing dosing solutions from the original undiluted stock solution with its higher ^{10}Be enrichment. This increase in sensitivity would enable tracing Be in cellular systems and for doing long-term toxicokinetic studies.

The ^{10}Be radioactivity levels used in this study are also worth noting. For the 0.5 µg ^{9}Be dose, each mouse received 9.9×10^{-4} GBq of ^{10}Be, as compared to 1.9×10^{5} GBq $^{7}BeCl_2$ used in the study by Sakaguchi **(**12**)**. Similarly, Lindenschmidt (21) used 1.1×10^{5} GBq of $^{7}BeCl_2$ in rats to investigate Be tissue distribution 48 hr after iv injection.

The 12% intrasample CV of biological samples by AMS is similar to the 9% CV determined in another AMS study for measuring ^{3}H in biological samples but higher than 2% reported for a new high throughput method for quantifying ^{14}C by AMS (11, 22). In addition, the larger intersample CV as seen in the liver tissue, demonstrates the inherent

variability that exists between mice in addition to the variability in the sample preparation procedure and the AMS measurements. Specifically, the CV calculated for the different blanks and standards was less than 2% and the CV of spiked tissues and dosed pooled samples was below 13% except for the femurs and lung measurements which were highly variable. We were not able to determine if the variability in the lung was due to differences in Be uptake from the intraperitoneal injection mode of administration or if the extraction procedure was not robust enough for this particular tissue. After the acid digestion step, the lung samples contained particulate material and it is possible that the observed variability is from incomplete oxidation of the organic material in the sample. This tissue might require a stronger oxidizer during the acid digestion step. For the femurs, on the other hand, a phosphomolybdate precipitation step was used to separate possible interfering inorganics found in bone such as calcium but this only lowered the CV from 74% (unpublished data, Lawrence Livermore National Laboratory, 2001) to 51% and is still much higher than the CV of other tissues. Additional method development is required for these tissues.

We assessed AMS performance and possible contamination from sample processing by comparing ratios obtained from carrier, process, and tissue blanks since Be occurs in the environment at only trace levels and the addition of ^{9}Be carrier solution to the samples is required (8). Some commercial ^{9}Be solutions can contain a ^{10}Be/^{9}Be ratio of ~5 x 10^{-14} (23) but our carrier blanks had lower ratios of ~1 x 10^{-14}. The different types of procedural blanks used in this study were not different from each other and provided a ^{10}Be/^{9}Be ratio of accelerator background (8).

Distribution of Be in Biological Tissues. Distribution of ^{10}Be in tissues dosed with 0.5 µg ^{9}Be was similar to that measured by Sakaguchi (12). Total body Be recovered from mice was 76 ± 5% of the initial dose, compared to the corresponding Sakaguchi results of 63 ± 2%. We consistently measured the highest concentrations of Be in the spleen followed by the liver with lower amounts in the other tissues. For example, the liver and spleen had significantly higher Be concentrations ($p=0.0006$ and $p<0.0001$, respectively) than the other tissues at the 0.5 µg dose. Sakaguchi observed the same distribution with both liver and spleen having the highest levels of Be compared to femurs and kidneys (blood and lung were not measured). Lindenschmidt (21) also measured highest levels of Be in liver and spleen of rats 2 days after ^{7}Be iv administration. High levels of Be in these organs is due to rapid absorption occurring in the peritoneal cavity from intraperitoneal injection (24). Infact, compounds are absorbed primarily through the portal circulation and therefore pass through the spleen and then the liver before reaching other organs (24). Sakaguchi (12) speculated that ^{7}Be administered by intraperitoneal injection is attached to the faces of the liver and spleen in the abdomen of the mice and then incorporated. They also suggest that concentration differences measured in the different tissues is due to differences in Be attachment to the organs besides differences in metabolism of Be. Elsewhere, it has been reported that the liver has a storage function for Be and that Be also accumulates in tissues of bones (18, 25).

The sensitivity of the AMS method was demonstrated by using animal doses that were 10 and 50-fold (0.001 and 0.05 µg) lower than the dose used in the Sakaguchi study. However, even though the spleen had high concentrations of Be compared to the other tissues, Be was not detected at the 1.0 ng dose because of the low instrument signal from

the small sample size (0.09 g) combined with the 250 µg of ^{9}Be carrier. In contrast, ^{10}Be/^{9}Be ratios were measured in livers at this low dose since about 20-fold more mass was used in the extraction.

Applications for ^{10}Be AMS in CBD Research. The AMS method described here is currently the most sensitive method for tracing beryllium in biological systems. The sensitivity obtained is applicable to a variety of potential studies of Be toxicity such as studies on Be complexation and reactions with macromolecules. ^{10}Be AMS in CBD research has the potential for tracing the binding of Be to cellular constituents such as the iron transport protein, ferritin or Be interactions with other cellular proteins (6). Other potential applications of this analytical tool also include animal inhalation studies and long-term tracer toxicokinetic studies.

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Acknowledgments

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Table 1. Be Doses ($^{10}Be/^{9}Be$ Ratio of 2.2×10^{-6}) Administered to Male ICR Mice by Intraperitoneal Injection and Tissues Removed from each Group

group #	*N*	Be dose (µg)[a]	tissues removed/group	other samples removed/group
1	10	0.05	spleen, lung, liver	
	3[b]	—		
2	5	0.001	kidney,lung, liver, femurs, spleen, blood	
	5	0.05		
	5	0.50		
	5	5.0		
	3[b]	—		
3	4	0.5		whole mouse, urine

[a]doses are equivalent to 0.03, 1.7, 17 and 170 µg/kg body weight
[b]mice dosed with 0.1 M phosphate buffer pH 7.9

Table 2. Wet Weight of Tissues

tissue	wet weight (g ± SD) [a]
kidney	0.49 ± 0.06
lung	0.31 ± 0.04
femurs	0.15 ± 0.04
spleen	0.09 ± 0.02
liver[a]	2.04 ± 0.21
blood	0.77 ± 0.16

[a] Average of five samples

Table 3. Expected and Measured $^{10}Be/^{9}Be$ Ratios of LANL and LLNL Standards[a]

Standard	Expected $^{10}Be/^{9}Be$ Ratio[b]	Measured $^{10}Be/^{9}Be$ Ratio
LANL (*N*=3)	1.1×10^{-10}	$(1.1 \pm 0.01) \times 10^{-10}$
	1.1×10^{-11}	$(1.1 \pm 0.01) \times 10^{-11}$
	1.1×10^{-12}	$(1.1 \pm 0.1) \times 10^{-12}$
	1.1×10^{-13}	$(1.0 \pm 0.02) \times 10^{-13}$
	6.0×10^{-14}	$(5.1 \pm 0.19) \times 10^{-14}$
LLNL (*N*=4)	1.0×10^{-11}	$(1.1 \pm 0.02) \times 10^{-11}$
	3.0×10^{-12}	$(3.0 \pm 0.03) \times 10^{-12}$
	1.0×10^{-12}	$(9.9 \pm 0.4) \times 10^{-13}$

[a]after addition of 1.0 mg ^{9}Be carrier

Table 4. Expected and Measured $^{10}Be/^{9}Be$ Ratios of Three Biological Matrixes Spiked with Different ^{9}Be Doses and $^{10}Be/^{9}Be$ Ratios

sample matrix[a]	^{9}Be Dose (µg/g tissue)	$^{10}Be/^{9}Be$ Ratio Dose	Expected $^{10}Be/^{9}Be$ Ratio[b]	Measured $^{10}Be/^{9}Be$ Ratio
liver	0.1	2.2×10^{-6}	2.2×10^{-11}	$(2.9 \pm 0.3) \times 10^{-11}$
	0.1	1.1×10^{-7}	1.1×10^{-12}	$(1.2 \pm 0.1) \times 10^{-12}$
	0.1	1.1×10^{-8}	1.1×10^{-13}	$(1.3 \pm 0.3) \times 10^{-13}$
	1.0	1.1×10^{-7}	1.1×10^{-11}	$(1.1 \pm 0.1) \times 10^{-11}$
	1.0	1.1×10^{-8}	1.1×10^{-12}	$(1.2 \pm 0.1) \times 10^{-12}$
	10	1.1×10^{-8}	1.1×10^{-11}	$(1.2 \pm 0.1) \times 10^{-11}$
	10	1.1×10^{-9}	1.1×10^{-12}	$(1.3 \pm 0.1) \times 10^{-12}$
lung	1.0	1.1×10^{-8}	2.2×10^{-12}	$(2.1 \pm 0.1) \times 10^{-11}$
urine	1.0	1.1×10^{-8}	1.1×10^{-12}	$(1.2 \pm 0.1) \times 10^{-12}$

[a]average sample size of liver and lung was 0.12 ± 0.02 g and 2.0 mL of urine (*N*=3)
[b]after addition of 1.0 mg ^{9}Be carrier

Table 5. Measured $^{9}Be/^{10}Be$ Ratios for Different Blank Types

Blank type	Measured $^{9}Be/^{10}Be$ ratio	CV (%)
Process (*N*=9)	$(16 \pm 0.3) \times 10^{-15}$	2
^{9}Be Carrier (*N*=4)	$(12 \pm 0.2) \times 10^{-15}$	1
Tissue (*N*=3)		
blood	$(17 \pm 2) \times 10^{-15}$	10
spleen	$(14 \pm 1) \times 10^{-15}$	6
liver	$(21 \pm 1) \times 10^{-15}$	5
kidney	$(10 \pm 1) \times 10^{-15}$	2
lung	$(12 \pm 3) \times 10^{-15}$	23
femurs	$(23 \pm 12) \times 10^{-15}$	51

Table 6. Intercepts and Slopes to Linear Fit with Significant Inequalities[a]

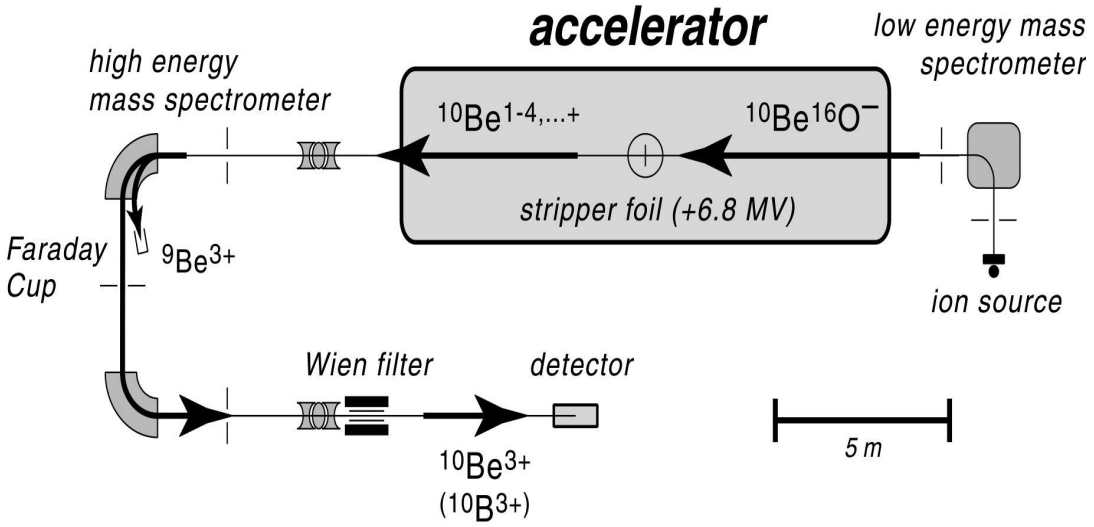
tissue	intercept value	A[b]	B	slope value	A	B
lung	-2.70	+		1.29		+
blood	-2.05	+		0.86	+	+
liver	-1.82	+		1.13		+
kidney	-1.53	+	+	0.76	+	
femur	-0.99		+	0.68	+	
spleen	-0.31		+	0.89	+	+

[a]0.05 level
[b]The "+" sign under the columns marked "A" and "B" show the sets of intercepts and slopes that were not significant.

Figure 1. Schematic of the Tandem Van de Graaf accelerator showing the major components for measuring $^{10}Be^{+3}$ and $^{9}Be^{+3}$.

Figure 2. Measured and expected $^{10}Be/^{9}Be$ ratios of serially diluted LANL and LLNL standards and spiked liver, urine and lung samples analyzed by AMS. Refer to Tables 3 and 4 for specific doses for each sample type. The data points represent one measurement per sample ± measurement error. The dashed line shows the LOD.

Figure 3. Log_{10} Be concentration in the liver, spleen, femurs, lung, kidneys and blood tissues of mice dosed with 0.001, 0.05, 0.5 and 5.0 µg Be (N = 5 per tissue).


accelerator
low energy mass spectrometer
high energy mass spectrometer
$^{10}Be^{1-4,...+}$
$^{10}Be^{16}O^{-}$
stripper foil (+6.8 MV)
Faraday Cup
$^{9}Be^{3+}$
ion source
Wien filter
detector
$^{10}Be^{3+}$
($^{10}B^{3+}$)
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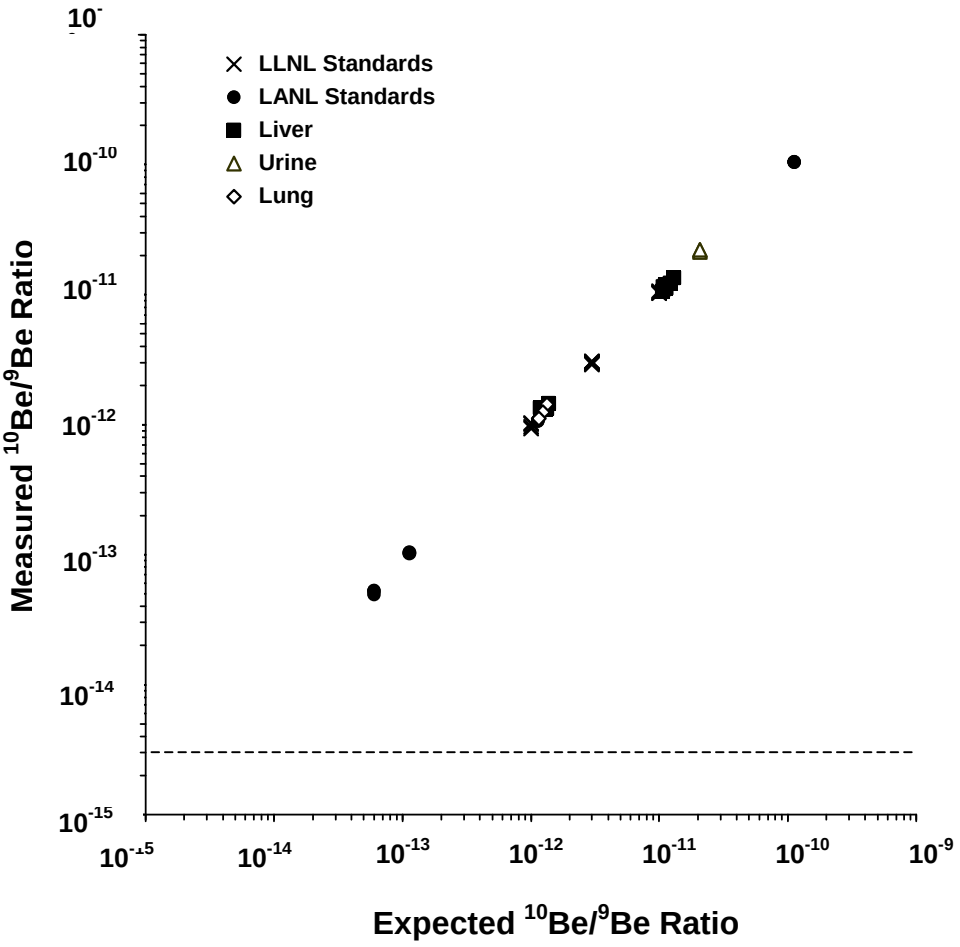
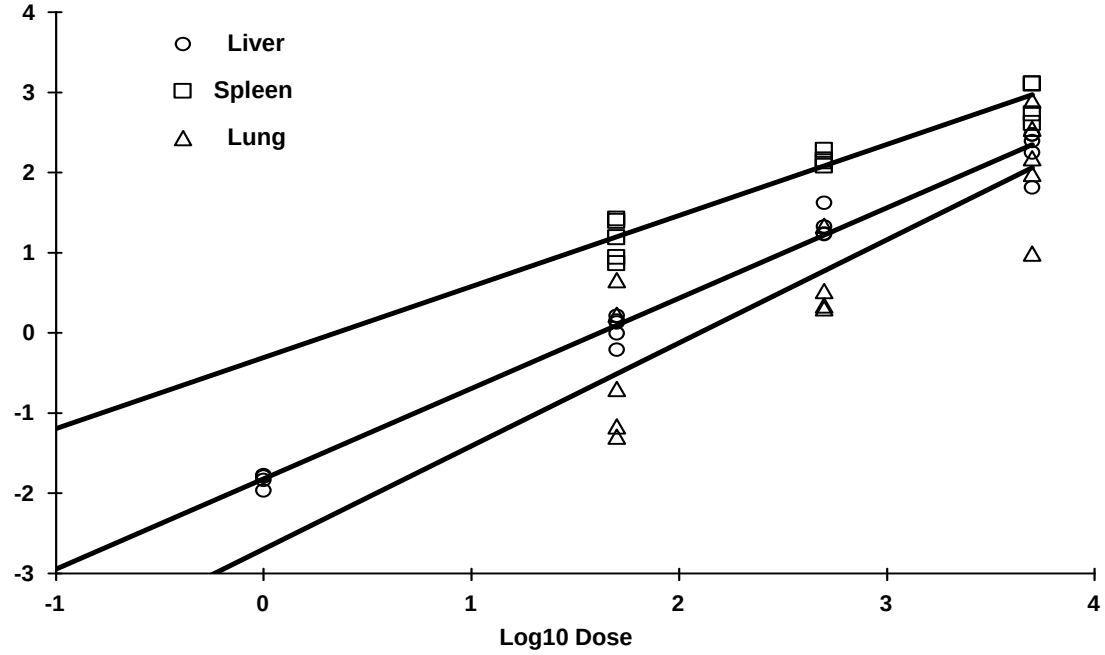
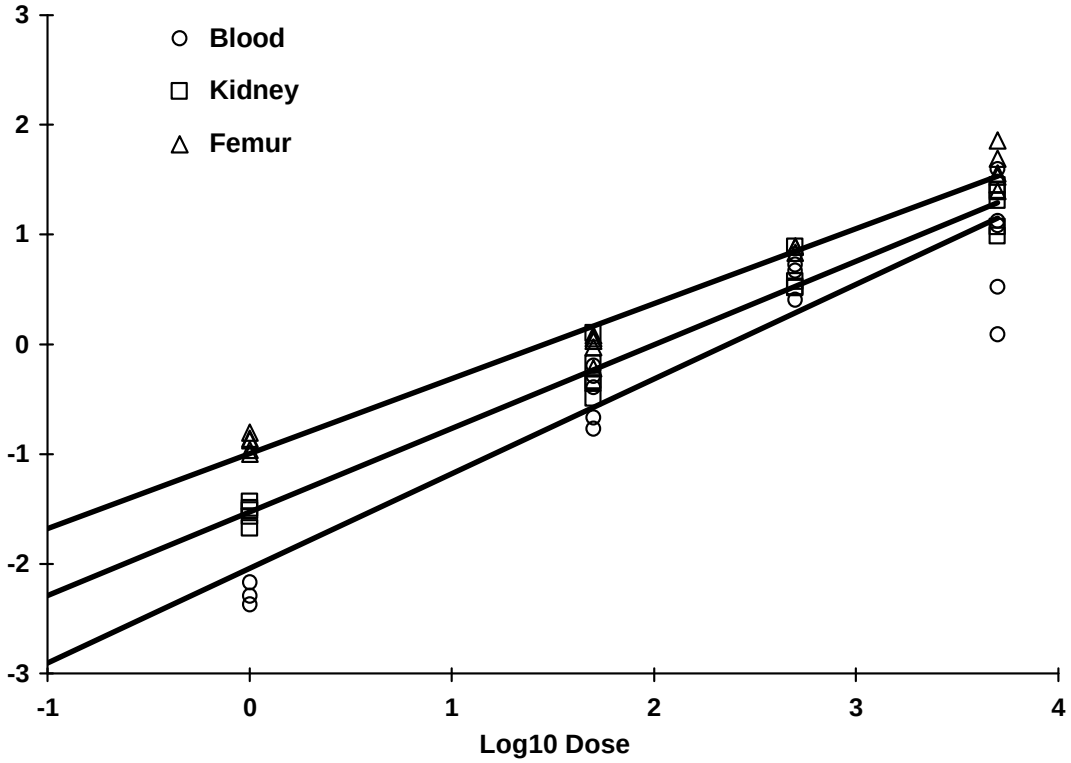

LLNL Standards
LANL Standards
Liver
Urine
Lung
Measured $^{10}Be/^{9}Be$ Ratio
Expected $^{10}Be/^{9}Be$ Ratio
10^{-10}
10^{-11}
10^{-12}
10^{-13}
10^{-14}
10^{-15}
10^{-9}


Liver
Spleen
Lung
4
3
2
1
0
-1
-2
-3
-1
0
1
2
3
4
Log10 Dose


Blood
Kidney
Femur
3
2
1
0
-1
-2
-3
-1
0
1
2
3
4
Log10 Dose